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**BRIEF REPORT****Improved molecular diagnosis of the common recurrent intragenic deletion mutation in *IKBKG* in a Filipino family with incontinentia pigmenti**

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## ABSTRACT

Incontinentia pigmenti is a rare multisystem X-linked dominant genetic disorder caused by mutations in *IKBKG*, encoding inhibitor of kappa light polypeptide gene enhancer in B-cells. Almost 80% or all cases result from a recurrent intragenic deletion mutation that removes exon 4-10 deletion. At present, this mutation can be detected by a multi-primer polymerase chain reaction (PCR) technique although current protocols may preferentially amplify the wild-type allele and may miss the deletion. Here, we report a female infant with incontinentia pigmenti that also affected her mother and sister, and two spontaneously aborted male siblings. We developed a modified PCR amplification method that provides more robust detection of the exon 4-10 deletion mutation, which was demonstrated in all affected females in this pedigree.

**Keywords:** X-linked, Blaschko, vesicle, mosaic, gene deletion

## INTRODUCTION

Incontinentia pigmenti (IP; MIM308300), also known as Bloch-Sulzberger syndrome, is an X-linked dominant disorder, with variable dermatological, ocular, skeletal, dental and neurological manifestations.<sup>1</sup> Rarely, cleft lip and palate can also occur.<sup>2</sup> Affected males almost always die prenatally, but cells expressing the mutated X

chromosome are eliminated selectively around the time of birth, and thus females with IP usually survive and exhibit extremely skewed X-inactivation. Clinically, the prominent cutaneous signs of IP occur in four classic phases: perinatal inflammatory erythema, vesicles and pustules, followed by verrucous papules and hyperkeratosis, and then hyperpigmentation, and subsequently, skin pallor, dermal atrophy and scarring.<sup>3</sup>

In 2000, the International Incontinentia Pigmenti Consortium demonstrated that IP results from mutations in *NEMO*, encoding NF-kappa-B essential modulator (NF- $\kappa$ B), which later became known as inhibitor of nuclear factor kappa-B kinase subunit gamma (IKK- $\gamma$ ) with the gene referred to as *IKBKG*.<sup>4</sup> The role of IKK- $\gamma$  is to regulate NF- $\kappa$ B activation of several genes involved in inflammation and cell survival, and thus in IP the lack of active NF- $\kappa$ B makes cells more prone to apoptosis.<sup>4</sup> The most common pathogenic mutation in *IKBKG* in patients with IP is a large intragenic deletion that removes exons 4-10, inclusive.<sup>5</sup> This single mutation accounts for ~80% of the total molecular pathology of IP and thus accurate methods to detect it are vital for disease diagnostics. Technically, this is not straightforward as there is a non-functional copy of *IKBKG* running in the opposite direction,<sup>6</sup> which may also show deletions with no phenotypic consequences, and thus new polymerase chain reaction (PCR) primer protocols have been developed,<sup>6</sup> and then further modified,<sup>7</sup> to address this potential concern. Although the newer protocols allow for both internal controls and false negatives,<sup>7</sup> there remains some bias in PCR amplification for the wild-type over the deletion mutant allele, which we address in this report. We also present the clinical features and molecular pathology of IP in a Filipino family with 5 affected members.

## CASE

The proband is a 24-day-old girl born at full term to unrelated parents. Full details of the pedigree are shown in Fig. 1a (the proband is individual III-5). Clinically, there were linear streaks of vesicles following Blaschko's lines, on the trunk, back and extremities but which spared the face, palms and soles (Fig. 1b). Bilateral clefting abnormalities affecting the lip and extending to the nose and palate were noted. A patch of hair loss was also seen on the scalp vertex. The rest of the physical examinations were unremarkable. Her mother (now aged 31 years) and an older sister (now 4 years) had similar perinatal pathologies. Notably, they both presented with linear vesicles at birth, which then evolved into Blascho-linear verrucous plaques. These hyperkeratotic lesions were then replaced by hyperpigmented linear discoloration and currently the skin manifestations are residual hypopigmented linear patches devoid of skin appendages. She has one older healthy brother (now aged 5 years) but her mother also reported spontaneous abortions of two male fetuses between 24-28 weeks' gestation. No abnormalities were reported in her father or other paternal or maternal relatives.

Laboratory tests did not identify any haematological or biochemical abnormalities. Following informed consent, a skin biopsy of a vesicle sampled under local anaesthetic revealed eosinophilic spongiosis with scattered, single necrotic keratinocytes in the epidermis and a moderately dense eosinophilic infiltrate in the

superficial dermis, consistent with the vesicular stage of IP (Fig. 2a). To determine whether the pathogenic mutation in *IKBKG* was the most frequently observed large intragenic deletion, we developed a modified version of a published protocol,<sup>7</sup> using the three primers detailed in that paper, but changing the buffer and amplification conditions because the existing protocol favours amplification of the wild-type allele because of large differences in the GC content of the PCR products: the 733-bp fragment (internal control) has a 56% GC content, whereas the 1045-bp deletion fragment has a 62% GC content. Presence of the deletion by PCR was demonstrated in the proband as well as the affected sister and mother (Fig. 2b) and was confirmed in the proband's DNA using L2Rev to Sanger sequence the 1045-bp fragment (Fig. 2c). Full details of the new recommended PCR protocol are presented in Figure 2d.

Most cases of IP are sporadic, with new mutations usually being paternal rather than maternal in origin.<sup>8</sup> Familial IP occurs in up to 25% of cases, with reports of up to four generations manifesting IP.<sup>9</sup> With regard to genotype-phenotype correlation, there is widespread clinical heterogeneity even within those individuals whose IP results from the recurrent *IKBKG* deletion, findings which reflect variable patterns and degrees of X-inactivation. Indeed, only the proband in this study has central facial abnormalities. Of most phenotypic concern in IP are potential central nervous system abnormalities, which can include developmental delay, seizures, hemiplegia, hemiparesis, spasticity, microcephaly and cerebellar ataxia.<sup>10</sup> None of these, however, manifested in any individuals in this Filipino family.

## CONCLUSION

We report a neonate with vesicular stage IP, whose mother and sister have IP too; two affected male siblings also died *in utero*. Clinically, these familial cases highlight the progressive stages of IP as it evolves. Using a modified PCR method we demonstrated presence of the recurrent exon 4-10 deletion mutation in *IKBKG* in all three living individuals with IP. This improved protocol delivers robust detection of this mutant allele and can be recommended for use in other laboratories.

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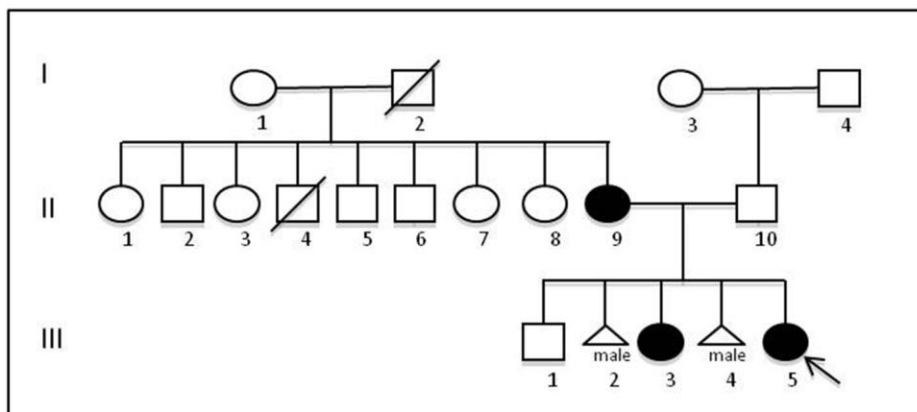


### Figure legends

**Figure 1.** Pedigree and clinical features in the proband. (a) The pedigree shows that the affected mother (II-9) has 5 offspring, four of whom have IP, including two male fetuses (III-2 and III-4) that were spontaneously aborted at 24 and 28 weeks gestation. (b) Blaschko-linear vesicles on the trunk and limbs in the proband (III-5) at 3 weeks of age. There is also cleft lip and palate and alopecia affecting the scalp vertex.

**Figure 2.** Skin and molecular pathology. (a) Light microscopy of lesional skin reveals eosinophilic spongiosis, collections of eosinophils within the upper epidermis, vesicle formation and haemorrhage (haematoxylin and eosin; bar = 25  $\mu$ m); (b) PCR amplification of peripheral blood genomic DNA identifies the recurrent intragenic deletion in *IKBKG* (for these specific PCR conditions the upper 1045-bp band indicates presence of the deletion, whereas the 733-bp band is wild-type) in the proband (III-5), and affected sister (III-3) and affected mother (II-9) but not control (Co) (MW = molecular weight marker); (c) Sanger sequencing of the 1045-bp band from the proband's amplified DNA confirms a breakpoint in intron 3/10 that deletes exon 4-10, inclusive; (d) details of the changes made to the PCR protocol for optimal detection of this internal deletion mutation.

(a)



(b)



